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(54) **Organic amine phosphonic acid complexes for the treatment of calcific tumors.**

(57) Particle-emitting radionuclides, eg. Samarium–153, have been complexed with phosphonic acid derivatives of organic amine or substituted organic amine compounds wherein the nitrogen and phosphorus are interconnected by an alkylene group. These complexes have been found useful in the treatment of calcific tumors in animals.

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ORGANIC AMINE PHOSPHONIC ACID COMPLEXES FOR THE TREATMENT OF CALCIFIC TUMORS

Aminophosphonic acids are known to chelate metal ions. Particularly stable chelates are formed with metals from the alkaline earth and transition metal series.

The development of a bone metastasis is a common and often catastrophic event for a cancer patient. The pain, pathological fractures, frequent neurological deficits and forced immobility caused by these metastatic lesions significantly decreases the quality of life for the cancer patient. The number of patients that contract metastatic disease is large since nearly 50% of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastases. This indicates the prevalence of the disease. Bone metastases are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix and other tumors, but collectively, these represent less than 20% of patients who develop bone metastases. Metastatic bone cancer is rarely life threatening and occasionally patients live for years following the discovery of the bone lesions. Initially, treatment goals center on relieving pain, reducing requirements for narcotic medication and increasing ambulation. Clearly, it is hoped that some of the cancers can be cured.

The use of radionuclides for treatment of cancer metastatic to the bon dates back to the early 1950's. It has been proposed to inject a radioactive particle-emitting nuclide in a suitable form for the treatment of calcific lesions. It is desirable that such nuclides be concentrated in the fast growing portion of the bone with minimal amounts reaching the soft tissue and normal bone. Radioactive phosphorus compounds (P-32 and P-33) have been proposed. However, the nuclear and biolocalization properties limit the use of these compounds. (Kaplan, E., et al, Journal of Nuclear Medicine, Vol. 1, No. 1, page 1, 1960); (U.S. Patent 3,965,254).

Another attempt has been made using phosphorus compounds containing a boron residue. The compounds were injected into the body (intravenously) and accumulated in the skeletal system. The patient was then irradiated with neutrons in order to activate the boron and give a therapeutic radiation dose. (U.S. Patent 4,399,817).

In the above-mentioned procedures, it is not possible to give therapeutic doses to the tumor without substantial damage to normal tissues. In many cases, especially for metastic bone lesions, the tumor has spread throughout the skeletal system and amputation or irradiation of a portion of the body is not practical. (Seminars in Nuclear Medicine, Vol. IX, No. 2, April, 1979).

The use of diphosphonate complexed with Re-186 has also been proposed. (Mathieu, L. et al, Int. J. Applied Rad. & Isotopes, Vol. 30, pp. 725-727, 1979; Weinenger, J., Ketring, A. R., et al, Journal of

Nuclear Medicine, Vol. 24, No. 5, page 125, 1983). However, the preparation and purification needed for this complex limits its utility and wide application.

Strontium-89 has also been proposed for patients with metastic bone lesions. However, the long half-life (50.4 days), high blood levels and low lesion to normal bone ratios limit the utility. (Firusian, N., Mellin, P., Schmidt, C. G., The Journal of Urology, Vol. 116, page 764, 1976; Schmidt, C. G., Firusian, N., Int. J. Clin. Pharmacol., 93:199-205, 1974).

A palliative treatment of bone metastases has been reported which employed I-131 labelled α-amino-(3-iodo-4-hydroxybenzylidene)diphosphonate (Eisenhut, M., Journal of Nuclear Medicine, Vol. 25, No. 12, pp. 1356-1361, 1984). The use of radioiodine as a therapeutic radionuclide is less than desirable due to the well known tendency of iodide to localize in the thyroid. Eisenhut lists iodide as one of the possible metabolites of this compound. In addition, any I-131 left over from the iodination reaction and not separated in the washing procedure also constitutes a threat to the thyroid. Indeed Eisenhut finds 0.1% of the injected dose present in the thyroid 24 hours after injection. The high energy of the gamma ray emission from I-131 leads to inferior quality images.

Therapeutically useful complexes according to the present invention must fit certain criteria insofar as possible. It should be recognized that there are many ligands, or complexing agents, which are included by our definition of the aminophosphonic acids in which the nitrogen of the amine and the phosphorus of the

phosphonic acid group are separat d by an alkylene group. Many may also contain other functional groups as substituents for some, but not all, of the amine hydrogens of the ligand. It should also be recognized that the properties of the particular isotope are important. The disadvantage of any one property may be overcome by the superiority of one or more of the properties of either ligand or isotope and their combination as the complex must be considered in toto.

The following is a discussion of the criteria which must be considered in choosing any particular combination of radioisotope and ligand.

Firstly, the complex must be taken up preferentially by the bone rather than by soft tissue. Most particularly, uptake in neither liver nor bone marrow is desired. Uptake by muscle tissue, while not as damaging, is also undesirable, resulting in "inferior" imaging properties as well as unwanted dosage to non-target organs.

Another important criterion is the ratio of the amount of complex taken up by the cancerous bone to that by normal bone. High ratios are preferred since it is desired to treat the cancerous bone while irradiating the normal bone as little as possible.

The complex should be cleared from the blood rapidly for obvious reasons.

With respect to the complexed radionuclide, considerations of the nuclear properties are essential. First, the half-life must be sufficiently long to allow for localization in the bone without delivering excessive

doses to non-target organs. When the complex is able to biolocalize rapidly, isotopes having shorter half-lives are useful. Isotopes with shorter half-lives provide an advantage since this permits the total dose to be given fractionally, allowing any non-target organs damaged by the radiation to recover between doses.

While for successful treatment of tumors the isotope must have sufficient particle emission, it is desirable, in order to quantify the localization of the complex, that the isotope also have sufficient gamma ray production that imaging is possible. The preferred gamma ray energy should be in the 100 to 200 keV (16×10^{-15} to 32×10^{-15} joule) range although some isotopes having higher energies are potentially useful. The amount of gamma radiation should be sufficient to image, but not so great that the patient needs to be isolated to prevent him from becoming a source of radiation exposure to persons near him.

There is a need, therefore, for a system possessing the above criteria by which it is possible to deliver therapeutic radiation doses to calcific tumors with minimal doses to soft tissue or normal bone.

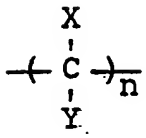
Such a system has now been found wherein a radioactive metal ion is complexed to a phosphorus-containing ligand. Certain of these complexes have been shown to be very selective for the skeletal system with very low soft tissue uptake. The material not taken up by bone is efficiently cleared through the kidneys into the bladder. The complexes also tend to

conc ntrate in areas of fast-growing bone much more readily than in normal bone. The radionuclides us d ar particle-emitting and a high radiation dose is deliver d in the area wher they ar deposited. Thus, therapeutic radiation doses can be delivered specifically to calcific tumors.

Particle-emitting radionuclides, e.g. Samarium-153, Yb-175, Lu-177 and Gd-159, have been complexed with organic amine or substituted organic amine phosphonic acid derivatives wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene group. These complexes have been found useful in the treatment of calcific tumors in animals.

The proposed use for the complexes of this invention is the therapeutic treatment of calcific tumors. These include primary tumors, where the skeletal system is the first site of involvement, and metastic bone cancer where the neoplasm spreads from other primary sites, e.g. prostate, breast, into the skeletal system. This invention provides a method of alleviating pain by delivering a therapeutic radiation dose to the aforementioned calcific tumors. This invention also provides a means of reducing the size of or destroying the calcific tumors by delivering a therapeutic radiation dose.

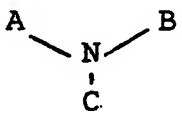
The organic phosphonic acid derivatives which have been found useful in complexing the particle-emitting radionuclides are organic amine or substituted organic amine compounds wherein the nitrogen and phosphorus are interconnected by an alkylene or substituted alkylene group having the formula



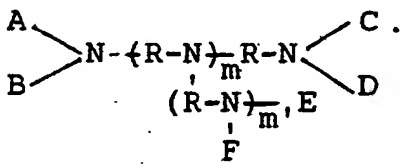
wherein X, Y are independently selected from hydrogen, hydroxyl, carboxyl, phosphonic, and hydrocarbon radicals having from 1-8 carbon atoms and physiologically acceptable salts of the acid radicals and n is 1-3 with the proviso that when n >1, each X and Y may be the same as or different from the X and Y of any other carbon atom.

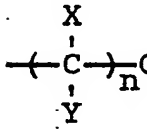
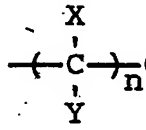
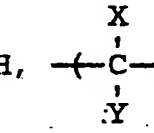
The compounds can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive N-H group with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof.

The following structural formulas represent some of the complexing ligands which can be used in the treatment of calcific tumors when complexed with a particle-emitting radionuclide:



and



wherein substituents A, B, C, D, E and F are independently selected from hydrogen,  OH,  COOH,  PO_3H_2, and physiologically acceptable salts of the acid radicals wherein X, Y, and n have been previously defined and m and m' each is 0-10 with the proviso that at least one of said nitrogen substituents is a phosphorus-containing

group and wherein R is a hydrocarbon residue which can be a linear, branched, cyclic, heterocyclic, substitut d heterocyclic, or a fused ring-type structure; with the further proviso that when m or m' >1 the E and F substituents may be the same as or different from any other substituent of any other nitrogen atom and each R can be the same as or different from any other R.

Methods for carboxyalkylating to obtain the amine derivatives containing one or more carboxyalkyl groups are well known (U.S. 3,726,912) as are the methods which give alkyl phosphonic and hydroxyalkyl (U.S. 3,398,198) substituents on the amine nitrogens.

Some specific, but non-limiting, examples of compounds which are included by the above structures are ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP), nitrilotrimethylenephosphonic acid (NTMP) and tris(2-aminoethyl)aminehexamethylenephosphonic acid (TTHMP).

Examples of particle-emitting nuclides useful in the practice of the invention are Sm-153, Yb-175, Lu-177 and Gd-159.

While Sm-153, Yb-175, Lu-177 and Gol-159 have been exemplified as the rare earth radionuclides employed with the complexing agents above, other rare earth radionuclides may also be complexed with the same aminophosphonic acid derivatives as disclosed herein. Representative, but non-limiting, example of such rare earth radionuclide is Ho-166.

The preferred embodiment of the present invention is a therapeutically effective complex of a particle-emitting radionuclide selected from the group consisting of gadolinium-159 (Gd-159), holmium-166 (Ho-166), lutetium-177 (Lu-177), samarium-153 (Sm-153) and ytterbium-175 (Yb-175) with an aminophosphonic acid derivative selected from the group consisting of ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP), nitrilotrimethylenephosphonic acid (NTMP) and tris(2-aminoethyl)amine-hexamethylenephosphonic acid (TTHMP).

For the purpose of convenience the abbreviations given in the parentheses above will be used to denote the respective radionuclides and aminophosphonic acid derivatives hereinafter.

A more preferred embodiment of the present invention is a therapeutically effective complex of a particle-emitting radionuclide selected from the group consisting of Gd-159, Lu-177, Sm-153, and Yb-175 with an aminophosphonic acid derivative selected from the group consisting of EDTMP, DTPMP, HEEDTMP, NTMP and TTHMP.

Particularly preferred embodiment of the present invention is a therapeutically effective complex of the particle-emitting radionuclide selected from the group consisting of Lu-177, Sm-153 and Yb-175 with an aminophosphonic acid derivative selected from the group consisting of EDTMP, DTPMP, HEEDTMP, NTMP and TTHMP.

The most preferred embodiment of the present invention is a therapeutically effective complex of Sm-153 with an aminophosphonic acid derivative selected from the group consisting of EDTMP, DTPMP, HEEDTMP, and TTHMP.

For the purpose of the present invention, therapeutically effective complexes described herein and physiologically-acceptable salts thereof are considered equivalent. Physiologically-acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the complex and which will not cause an adverse physiological effect when administered to an animal at dosages consistent with good pharmacological activity. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary amines and the like. Physiologically-acceptable salts of the present invention may be prepared by treating the complex having at least one acid group with an appropriate base.

Radionuclides can be produced in several ways. In a reactor, a nuclide is bombarded with neutrons to obtain a nuclide with additional neutrons in its nucleus.

e.g. Sm-152 + neutron $\longrightarrow$ Sm-153 + gamma

and complex. Although complexation will occur at other pH values, pH from 5-11 is preferred for complexation.

The metal and ligand may be combined under any conditions which allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of ligand and metal) is all that is required. In some cases heating may be required to obtain maximum complex yield.

The ratio of ligand to metal is a result of two competing considerations. As indicated above the ligand and metal are believed to be in equilibrium with the complex. On the one hand, it is desirable to have a large quantity of ligand (L), so that there is a minimum amount of free metal (M), because the uncomplexed metal may cause serious side effects in the patient. On the other hand, excess free ligand may compete for sites in the target, thus rendering the treatment less effective. The fact that the ligands within the scope of the invention are capable of complexing with varying molar ratios of metal adds a degree of complexity to these considerations. Although it is difficult to generalize, it is believed that the ligand to metal molar ratio is desirably 0.1:1 to 3000:1, preferably 1:1 to 2000:1, more preferably 1:1 to 1000:1.

The samarium used in the following example was either natural Sm_2O_3 (99.9 percent from Spex Industries) or isotopically enriched (99.06 percent Sm-152) Sm_2O_3.

The Sm-153 used in this study was produced by neutron irradiation at the University of Missouri

Research Reactor. Preliminary studies were carried out using Sm-153 produced by short (5-30 minutes) irradiations of natural Sm_2O_3, in the reactor's pneumatic tube system. The specific activity of Sm-153 produced by this method was 0.5-3.0 Ci/g (18.5 to 111 GBq/g).

The majority of this work was carried out using Sm-153 produced by irradiating 99.06 percent enriched $^{152}Sm_2O_3$ in the first row reflector at a neutron flux of 1×10^{14} neutron/cm^2·sec. Irradiations were generally carried out for 50-60 hours, yielding a Sm-153 specific activity of 1000 - 1300 Ci/g (37×10^3 to 48.1×10^3 GBq/g).

To irradiate Sm_2O_3 for production of Sm-153, the desired amount of target was first weighed into a quartz vial, the vial flame sealed under vacuum and welded into an aluminum can. The can was irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial was removed and transferred to a glove box, crushed into a glass vial which was then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1-4 M HCl was then added to the vial via syringe to dissolve the Sm_2O_3. Once dissolved, the solution was diluted to the appropriate volume by addition of water. The solution was removed from the original dissolution vial which contains the chards of the crushed quartz vial, and transferred via syringe to a clean glass serum vial. This solution was then used for complex preparation. Similar procedures were used to prepare other radionuclides, e.g. Lu-177, Yb-175, Gd-159.

The various complexes employed in this invention were prepared as follows: the desired amount of ligand was placed in a vial and dissolved by addition of water. At some higher ligand concentrations, it was necessary to add base in order to completely dissolve the ligand. Heating was also found to be useful for dissolving the ligands. The appropriate amount of the samarium or other radionuclides in the stock solution described above was then added to the ligand solution. The pH of the resulting solution was then raised to the appropriate level by addition of NaOH. The solution was heated to 60°-70°C for 30 minutes in a water bath to insure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

The complex yield was determined by placing 5-20 microliters (depending on activity) of the complex solution onto a 0.5 cm^3 column of a commercially available synthetic organic cation exchange resin. The column was then eluted with two separate 10 milliliter volumes of isotonic saline. The anionic complexes are not retained by the resin and were eluted by the saline solution, while any uncomplexed metal was retained on the column. The eluant solutions and the column were then counted for the characteristic emission of the particular radionuclide, e.g. 103 keV (16.5×10^{-15} joule) gamma ray of Sm-153. The complex yield was obtained by adding the counts in the eluant solutions and dividing by the total of the eluant solutions and the column.

This invention provides a means of delivering a therapeutic radiation dose to calcific tumors. It may also be desirable in the cases where the radionuclide has imageable gamma photons to inject a "sub-therapeutic"

dose and determine the fate of the radionuclide using a scintillation camera prior to injecting a therapeutic radiation dose. Therefore, the levels of radiation injected could be as low as 1 mCi (37 MBq) for imaging prior to the therapy. Therapeutic doses will be greater. The dose to the tumor may range from 100 to 10,000 rads (1 Gy to 100 Gy). The preferred dose to the tumor ranges from 1,000 to 8,000 rads (10 Gy to 80 Gy). For complexes such as Sm-153-EDTMP amounts ranging from 0.1 mCi/kg body weight to 3 mCi/kg body weight are preferred. The amount of activity required to deliver a therapeutic dose may vary with the individual radionuclides. Individual doses may be given in one injection or fractionated into several injections totaling the aforementioned dose per treatment.

Biodistribution of various complexes of the present invention was studied in rats and rabbits.

Studies to determine the qualitative dis--tribution of the various complexes of the present invention were conducted by injecting the complexes into rats and obtaining the gamma ray images of the entire animal at various times up to two hours after injection.

Male Spague Dawley rats were anesthetized by injection with sodium pentabarbitol and the jugular vein cannulated. The animals were then injected with 50 to 150 microliters of the various complexes and gamma ray images were taken on a Nuclear Chicago® Pho-Gamma® II scintillation camera immediately after injection and approximately every half hour thereafter for two hours.

Quantitative biodistributions were obtained by injecting 50-100 microliters of the complex solution into the tail vein of unanesthetized male Spague Dawley rats. The rats were then placed in cages lined with absorbent paper in order to collect all urine excreted prior to sacrifice. After two hours, the rats were sacrificed by cervical dislocation and the various tissues dissected out. The samples were then rinsed with saline, blotted dry on absorbent paper and weighed. The samples were counted with a NaI uptake counter approximately one foot from the face of the crystal, in order to minimize geometry differences between the different size samples.

To minimize differences in age between groups of animals, all rats used were in the 160-220 gram weight range. All animals were kept "in-house" for at least one week prior to use to minimize the effects of stress that occur during shipping.

The various complexes of the present invention were also evaluated in rabbits. The animals used in these studies were male New Zealand white rabbits in the 2.6 to 3.2 kilogram weight range. The animals were kept "in-house" for at least a week prior to use.

The rabbits were injected with 100-250 microliters of the complex solution via a cannula placed in the marginal ear vein. In studies where blood clearance was measured, blood samples were taken through a heparinized cannula placed in the marginal vein of the ear not used for injection of the complex.

Three hours after injection, a blood sample was taken by cardiac puncture and the animal was then

sacrificed by injection of a commercial euthanasia solution. After sacrifice, images were obtained by placing the carcass directly on the face of a large field of view scintillation camera.

Uptake of the complexes in the lesion compared to uptake in normal bone, or lesion/normal bone ratios, is a particularly important parameter for calculating the complex suitability as a therapeutic agent. To determine the lesion to normal bone ratios, a modified drill hole method (Subramanian, G. et al., 19th lit. Annual Meetings of S.N.M., Bern, Switzerland, September 8-11, 1981) was used.

In order to simulate uptake in rapidly growing bone, such as that found in cancerous bone, two holes were drilled into the surface of the tibia of a rabbit in order to damage the bone. Seven to ten days later, the animal was injected with the complex. After three hours, the animal was anesthetized and imaged using an Anger camera and pinhole collimator.

The following examples are illustrative of the present invention and are not to be construed as limiting the scope thereof.

Example 1

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (94.5 g) and degassed water (100 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydrochloric acid (112 ml). The dropping funnel was charged

with ethylenediamine (15 g) and adjusted to allow dropwise addition of the diamine to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of this time the dropping funnel was charged with formaldehyde (85 g of a 37% aqueous solution) which was added dropwise over a two-hour period with continued heating to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool slowly overnight during which time the product precipitated. Vacuum filtration followed by cold water washing yielded ethylenediaminetetramethylenephosphonic acid (EDTMP).

Example 2

25 to 35 milligrams of EDTMP prepared in Example 1 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this, 0.25 ml of Sm-153 (~ 10 mCi(~ 370 MBq) in dilute HCl was added. The pH of the resulting solution was then adjusted to 10 by addition of NaOH. The resulting solution was heated to 60°C - 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl. The yield of the complex was over 95 percent.

Laboratory rats were injected with the above complex (50-100 µℓ) via the tail vein. After 2 hours, the animals were sacrificed by cervical dislocation and organs and tissues removed. Samples were counted with a NaI uptake counter to determine the biolocalization of the complex. It was found that a significant amount (55-65 percent) of the activity was concentrated in th

skeletal system with very little soft tissue uptake. Most of th activity not found in the skeleton was cleared through the kidneys into the bladd r. Scintillation scans of animals treated in the same manner showed the activity concentrating in the skeletal system. The lesion to normal bone ratio (drill hole model) (This data was obtained by the method of Subramanian, G., McAfee, J. G., et al, 19th Int. Annual Meeting of S.N.M., Bern, Switzerland, September 8-11, 1981.) for this complex was approximately equal to that of Tc-99m-MDP (MDP refers to methylene diphosphonate), a commercially available diagnostic bone agent.

Example 3

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (94.5g) and degassed water (100 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydrochloric acid (112 ml). The dropping funnel was charged with diethylenetriamine (20.6 g) and adjusted to allow dropwise addition of the amine to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of this time the dropping funnel was charged with formaldehyde (85 g of a 37% aqueous solution) which was added dropwise over a two-hour period with continued heating to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool. Diethylenetriaminepentamethylenephosphonic acid (DTPMP) was isolated from the reaction mixture.

Example 4

20 to 30 milligrams of DTPMP prepared in Example 3 was weighed into a vial and dissolved using 0.75 ml of distill d water. To this, 0.25 ml of Sm-153 (~ 10 mCi(~ 370 MBq)) in dilute HCl was added. The pH of the resulting solution was then adjusted to 10 by addition of NaOH. The solution was then heated to 60°C-70°C for 30 minutes in a water bath to ensure maximum complex formation.

The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl. The yield of the complex was over 95 percent.

The above complex was tested in rats. The results in rats showed ~ 30 percent of the activity in the skeletal system with very little activity in any soft tissue.

Example 5

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (94.5 g) and degassed water (100 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydrochloric acid (112 ml). The dropping funnel was charged with N-hydroxyethylethylenediamine (34.6 g) and adjusted to allow dropwise addition of the diamine to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of this time the dropping funnel was charged with formaldehyde (85 g of a 37% aqueous solution) which was added dropwise over a two hour period with

continued heating to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool. Hydroxyethylethylenediamine-trimethylenephosphonic acid (HEEDTMP) was isolated from the reaction mixture.

Example 6

30 to 40 milligrams of HEEDTMP prepared in Example 5 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this, 0.25 ml of Sm-153 (~ 10 mCi(~ 370 MBq)) in dilute HCl was added. The pH of the resulting solution was then adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl. The yield of the complex was over 95 percent.

Example 7

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (57.7 g) and degassed water (50 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydrochloric acid (50 ml). The dropping funnel was charged with tris(2-aminoethyl)amine (13.7 g) and adjusted to allow dropwise addition of the amine to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of this time the dropping funnel was charged with formaldehyde (51 g of a 37% aqueous solution) which was added dropwise over a two-hour period with continued heating

to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool. Tris(2-aminoethyl)aminehexamethylenephosphonic acid (TTHMP) was isolated from the reaction.

Example 8

48 to 53 milligrams of TTHMP prepared in Example 7 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this solution, 0.25 ml of Sm-153 (~10 mCi(~ 370 MBq)) in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

The complexes of Examples 2, 4, 6 and 8 were tested in rats. The two-hour rat biodistribution data for these complexes is shown in Table I.

TABLE I

	Example Nos.			
	2	4	6	8
% Dose in Skeleton*	58 ±4	30 ±10	57 ±7	28 ±4
Blood	0.032 ±0.016	0.16 ±0.12	0.035 ±0.006	0.25 ±0.14
Liver	0.25 ±0.04	0.27 ±0.13	0.45 ±0.07	0.18 ±0.02
Urine	49 ±4	74 ±8	50 ±4	65 ±9
Bone/Blood	1800 ±1200	224 ±150	1300 ±160	80 ±20
Bone/Muscle	1500 ±500	220 ±130	1300 ±180	410 ±75

Number of rats tested = 5

*Based on % dose in femur X 25

Example 9

35 to 45 milligrams of EDTMP prepared according to Example 1 was weighed into a vial and dissolved using 0.75 ml of distilled water. To this solution, 0.25 ml of Yb-175 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 10

55 to 60 milligrams of DTPMP prepared according to Example 3 was weighed into a vial and dissolved

using 0.75 ml of distilled water. To this solution, 0.25 ml of Yb-175 in dilute HCl was added. The pH of the resulting solution was adjust d to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 11

50 to 55 milligrams of HEEDTMP prepared according to Example 5 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Yb-175 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes in a water bath to ensure maximum complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 12

Into a suitable reaction vessel equipped with a thermometer, magnetic stirring bar, dropping funnel, and an atmosphere of nitrogen were charged phosphorous acid (94.5 g) and degassed water (100 ml). Dissolution of the phosphorous acid was achieved by stirring and the solution was then treated with concentrated hydro--chloric acid (112 ml). The dropping funnel was charged with ammonium chloride (17.2 g in an aqueous solution) and adjusted to allow dropwise addition of the ammonium chloride to the acidic solution. When addition was complete a heating mantle was installed and the solution refluxed for one hour. At the end of this time the dropping funnel was charged with formadehyde (85 g of a 37% aqueous solution) which was added dropwise over

a two-hour period with continued heating to maintain reflux during the addition. After all the formaldehyde was added, the reaction mixture was stirred under reflux for an additional two hours, then allowed to cool, yielding nitrilotrimethylenephosphonic acid (NTMP).

Example 13

50 to 55 milligrams of NTMP prepared in Example 12 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Yb-175 in dilute HCl was added. The pH of the resulting solution was then adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Complexes of Examples 9, 10, 11 and 13 were tested in rats. The biodistribution data are shown in Table II.

TABLE II

	Example Nos.			
	9	10	11	13
% Dose in Skeleton*	50 ±7	25 ±2	56 ±2	63 ±5
Blood	0.074 ±0.027	0.116 ±0.040	0.231 ±0.044	0.204 ±0.059
Liver	0.138 ±0.036	0.112 ±0.027	0.214 ±0.082	0.236 ±0.072
Bone/Blood	562 ±130	179 ±60	190 ±40	256 ±90
Bone/Muscle	619 ±192	366 ±96	572 ±214	876 ±239

Number of rats tested = 5

*Based on % dose in femur X 25

Example 14

50 to 55 milligrams of EDTMP prepared in Example 1 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Lu-177 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C for 30 minutes to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 15

55 to 60 milligrams of HEEDTMP prepared according to Example 5 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this,

0.25 ml of Lu-177 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. Th solution was then heated between 60°C and 70°C for 30 minutes to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Complexes of Examples 14 and 15 were tested in rats. The biodistribution data are shown in Table III.

TABLE III

	Example Nos.	
	14	15
% Dose in Skeleton*	51 ±5	58 ±3
Blood	0.10 ±0.06	0.17 ±0.08
Liver	0.48 ±0.15	1.9 ±0.7
Kidneys	0.28 ±0.03	0.30 ±0.02
Muscle	0.44 ±0.27	0.56 ±0.16
Bone/Blood	500 ±220	370 ±320
Bone/Muscle	1380 ±1900	570 ±180

Number of rats tested = 5

* Based on % dose in femur X 25

Comparative Example

Two commercially available non-nitrogen-containing phosphonic acid compounds which are well known as diagnostically useful compounds when complexed with Tc-99m were complexed with Sm-153 and tested in the manner of the complexes of the present invention. The data comparable to that given for the examples of the invention is shown in Table A below:

TABLE A. Two Hour Biodistribution of Complexes in Rats

	^{153}Sm-MDP***	^{153}Sm-HEDP*
% Dose in Skeleton**	1.65 ±0.43	20.5 ±1.4
Blood	0.23 ±0.19	13.1 ±2.6
Liver	84.5 ±3.8	3.5 ±0.9
Kidney	0.45 ±0.19	0.99 ±0.44
Urine	0.20 ±0.14	41.3 ±4.6
Bone/Blood	8.6 ±5.0	1.3 ±0.3
Bone/Muscle	7.1 ±3.2	10.5 ±2.9

Number of rats tested = 5

* 1-hydroxyethylidine-1,1-diphosphonic acid (HEDP)

** Based on % dose in femur X 25

***Methylenediphosphonate

It should be noted that the comparative examples in above Table A ar of two non-nitrogen-containing phosphonic acid complexes. These have certain undesirable properties which make them inferior to the complexes of the invention. Thus, the Sm-MDP shows high liver uptake while the Sm-HEDP has both low skeletal uptake and poor clearance from the blood.

Example 16

The complex tested on rats in Example 2 (Sm-153-EDTMP) was also tested in rabbits in a similar manner. Results of biodistribution (averaged for 5 rabbits) are summarized in Table IV.

TABLE IV

% Dose in Skeleton*	66 ± 5
Blood	0.12 ± 0.10
Liver	0.95 ± 0.42
Urine	34 ± 4
Bone/Blood	900 ± 800
Bone/Muscle	1200 ± 400

* Based on % dose in femur X 20.1.

Example 17

An English Setter with a tumor in his pelvis was injected with ~19 mCi (700 MBq) of the complex of Example 2 (Sm-153-EDTMP). After 2 hours, the dog was imaged using a scintillation camera. The scintillation scan showed high uptake of the complex in the tumor and looked very similar to an earlier scan performed using Tc-99m-MDP. The uptake ratio of the Sm-153 complex in

the tumor to normal bone was very similar to that of the Tc-99m complex. A scintillation scan of the dog five days after treatment gave similar results. Seven days after the treatment the dog appeared to be in l ss pain as evidenced by an increase in its activity.

Example 18

A series of rats was injected with the complex of Example 2 (Sm-153-EDTMP) and sacrificed at various intervals. The rat biodistribution data is summarized in Table V. This data shows rapid biolocalization properties, e.g. bone uptake and blood clearance, as well as permanent localization of the activity in the skeletal system.

Example 19

An Irish Setter with a tumor in the femur was injected with the complex of Example 2 (Sm-153-EDTMP). The leg was amputated and samples of the tumor and normal bone were counted for Sm-153. The amount of Sm-153 in the tumor was 15-20 times that of nonlesionous bone from the same leg.

Example 20

A series of 5 rabbits was injected with the complex of Example 2 (Sm-153-EDTMP). Less than 0.15 percent of the injected dose was found in the bone marrow.

TABLE V

BIODISTRIBUTION OF Sm-153-EDTMP OVER TIME IN RATS

ORGAN	% Administered Dose After							
	15 Min.	30 Min.	1 Hour	2 Hours	5 Hours	24 Hours	48 Hours	72 Hours
Skeleton*	48 ±4	53 ±2	58 ±3	58 ±4	59 ±4	52 ±3	60 ±1	57 ±4
Blood	5.852 ±.553	2.304 ±.476	.532 ±.395	.032 ±.016	.008 ±.019	.007 ±.002	.006 ±.001	.006 ±.001
Liver	.959 ±.137	.526 ±.108	.322 ±.073	.252 ±.038	.370 ±.080	.349 ±.021	.458 ±.079	.492 ±.115
Kidneys	1.745 ±.334	.805 ±.069	.466 ±.126	.254 ±.035	.364 ±.055	.250 ±.087	.286 ±.028	.216 ±.036
Urine	28 ±3	42 ±5	47 ±3	49 ±4	46 ±3	55 ±2	50 ±2	53 ±1
Bone/Muscle	30 ±2	70 ±30	300 ±200	1500 ±500	3600 ±1900	2400 ±600	2800 ±400	3400 ±800
Bone/Blood	7 ±1	20 ±4	120 ±60	1800 ±1400	4200 ±3700	6700 ±1400	8200 ±1600	7800 ±1600

Number of rats tested = 5

*Based on % dose in femur x 25

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Example 21

48 to 53 milligrams of EDTMP prepared according to Example 1 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Gd-159 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C in a water bath to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Example 22

55 to 60 milligrams of HEEDTMP prepared according to Example 5 was weighed into a vial and dissolved with 0.75 ml of distilled water. To this solution, 0.25 ml of Gd-159 in dilute HCl was added. The pH of the resulting solution was adjusted to 10 by addition of NaOH. The solution was then heated between 60°C and 70°C in a water bath to optimize complex formation. The pH of the solution was then adjusted to 7-8 by addition of 1 M HCl.

Complexes of Examples 21 and 22 were tested in rats. The biodistribution data are shown in Table VI.

TABLE VI

	% Dose in Example No. 21	% Dose in Example No. 22
Skeleton*	57 ±3	60 ±4
Blood	0.15 ±0.03	0.14 ±0.02
Liver	0.25 ±0.04	0.57 ±0.07
Kidneys	0.33 ±0.09	0.58 ±0.11
Muscle	0.56 ±0.15	0.76 ±0.42
Bone/Blood	305 ±42	335 ±35
Bone/Muscle	577 ±192	548 ±419

*Calculated from % dose femur x 25

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CLAIMS

1. A therapeutically effective compl x of a paticle-emitting radionuclide which is gadolinium-159 (Gd-159), holmium-166 (Ho-166), lutetium-177 (Lu-177), samarium-153 (Sm-153) or ytterbium-175 (Yb-175) with an aminophosphonic acid derivative which is ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEEDTMP), nitrilotrimethylenephosphonic acid (NTMP) or tris(2-aminoethyl) aminehexamethylenephosphonic acid (TTHMP) or a physiologically acceptable salt of such a complex.

2. A complex as claimed in Claim 1, characterized in that said radionuclide is Gd-159, Lu-177, Sm-153 and Yb-175.

3. A complex as claimed in Claim 2, characterized in that said radionuclide is selected from the group consisting of Lu-177, Sm-153 or Yb-175.

4. A complex as claimed in Claim 3, characterized in that said radionuclide is Sm-153.

5. A complex as claimed in Claim 3, characterized in that said radionuclide is Lu-177.

6. A complex as claimed in Claim 3, characterized in that said radionuclide is Yb-175.

7. A complex as claimed in Claim 2, characterized in

said radionuclide is Gd-159.

8. A complex as claim d in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is ethylenediaminetetramethylenephosphonic acid (EDTMP).

9. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is diethylenetriaminepentamethylene-phosphonic acid (DTPMP).

10. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is hydroxyethylethylenediaminetrimethylene phosphonic acid (HEEDTMP).

11. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is nitrilotrimethylenephosphonic acid (NTMP).

12. A complex as claimed in any one of Claims 1 to 7, characterized in that said aminophosphonic acid derivative is tris(2-aminoethyl) aminehexamethylene-phosphonic acid (TTHMP).

13. A process for the preparation of a therapeutically effective complex as claimed in Claim 1, characterized in that a radionuclide which is Gd-159, Ho-166, Lu-177, Sm-153, or Yb-175 is contacted with an aminophosphonic acid derivative which is

EDTMP, DTPMP, HEEDTMP, NTMP or TTHMP.

14. A process as claim d in Claim 13, wh rein th said contact is carried out in the presence of water at a pH of from 5 to 11.

15. A process as claimed in Claim 13 or Claim 14, wherein the said contact is carried out at elevated temperature.

16. A process as claimed in any one of Claims 13 to 15, including the step of adjusting the pH of the complex to from 7 to 8.

17. A process as claimed in any one of Claims 13 to 16, characterized in that said radionuclide is Gd-159, Lu-177, Sm-153 or Yb-175.

18. A process as claimed in Claim 17, characterized in that said radionuclide is Lu-177, Sm-153 or Yb-175.

19. A process as claimed in Claim 18, characterized in that said radionuclide is Sm-153.

20. A process as claimed in Claim 18, characterized in that said radionuclide is Lu-177.

21. A process as claimed in Claim 18, characterized in that said radionuclide is Yb-175.

22. A process as claimed in Claim 17, charaterized in that said radionuclide is Gd-159.

23. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid

derivative is EDTMP.

24. A proc ss as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is DTPMP.

25. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is HEEDTMP.

26. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is NTMP.

27. A process as claimed in any one of Claims 13 to 22, characterized in that said aminophosphonic acid derivative is TTHMP.

28. A process as claimed in any one of Claims 13 to 28, characterized in that said aminophosphonic and said radionuclide acid derivative are contacted in a molar ratio of from 0.1:1 to 3000:1.

29. A complex as claimed in any one of Claims 1 to 12, for use in the treatment of cancer.

30. The use of a complex as claimed in any one of Claims 1 to 12, in the manufacture of a medicament for the treatment of cancer.